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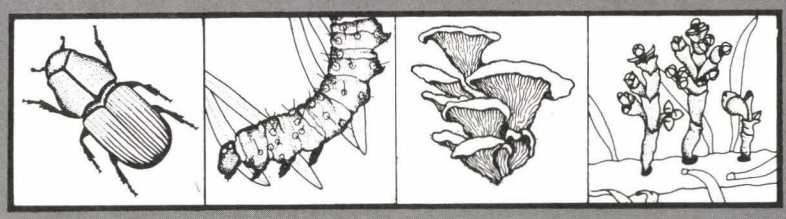

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ASSOCIATION OF FUSARIUM WITH NONDISEASED CONTAINERIZED PONDEROSA PINE SEEDLINGS AT THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

A total of 45 nondiseased containerized ponderosa pine seedlings from 10 seedlots were sampled at three different times during the growing season for root infection by *Fusarium* and *Trichoderma* at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. More than 90 percent of the seedlings had roots infected with *Fusarium*. Inverse relationships existed between amount of root infection by *Fusarium* and *Trichoderma*. Leach® pine cells in which seedlings were growing and soil adjacent to roots and on the inner wall surface of containers were also heavily colonized by *Fusarium*. *Fusarium oxysporum* was the most commonly encountered species of *Fusarium*. Despite extensive root colonization by *Fusarium*, seedling roots lacked lesions and no effects on seedling growth were detected.

INTRODUCTION

Fusarium spp. include important pathogenic organisms that may seriously impact conifer seedling production in forest tree nurseries. *Fusarium*-associated diseases are often most damaging on containerized seedlings in northern Rocky Mountain nurseries (James 1984a). Although disease may become evident throughout the growth cycle, manifestations of root diseases are often most pronounced at the end of the cycle when seedlings are stressed during bud setting (James 1986a; James, Gilligan and Reedy 1988). It has been hypothesized that many seedlings become infected with fusaria relatively early in their life but do not display disease symptoms until conditions are conducive for pathogenesis (James 1986a; James et al. 1987).

Past experience with *Fusarium*-associated diseases of containerized seedlings has indicated that Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is usually the most severely affected species (James 1984a; James 1986; James et al. 1987). Roots from Douglas-fir seedlings with very slight disease symptoms (foliar tip necrosis or needle chlorosis) are usually extensively colonized with *Fusarium* (James 1986b). Also, random samples of nondiseased Douglas-fir seedlings have indicated that many seedlings can be infected without displaying disease symptoms (James et al. 1987). Isolates of *Fusarium* from nondiseased seedlings have been shown to be pathogenic (James et al. 1988).

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Extensive efforts have been made to identify the most important sources of *Fusarium* inoculum for infection of containerized seedlings. Initially, infected seed was thought to be a major inoculum source (James 1983; James 1985c; James 1986a; James 1987b). Investigations have indicated that other possible sources include weeds within greenhouses (James et al. 1987), peat-vermiculite soil mixes (James 1985a; James and Gilligan 1984), and containers within which seedlings are grown (James and Gilligan 1988a; James, Gilligan and Reedy 1988). Recent evidence indicates that styroblock (James 1987c; James, Gilligan and Reedy 1988) and Leach® containers may both harbor fusaria even after they have been cleaned. Therefore, it appears probable that most containerized seedlings will be exposed to some level of *Fusarium* while they are being produced in nurseries.

To confirm this conclusion, investigations were conducted at the the USDA Forest Service Nursery in Coeur d'Alene, Idaho to determine extent of root infection by *Fusarium* on ponderosa pine (*Pinus ponderosa* Laws.) seedlings which appeared healthy and did not exhibit disease symptoms. Ponderosa pine was chosen because root disease is rare on this species at the nursery.

MATERIALS AND METHODS

Forty-five nondiseased ponderosa pine seedlings from 10 seedlots were randomly selected for sampling at three different times during the 1987 growing season (table 1). All selected seedlings appeared healthy and lacked any foliar symptoms indicative of root disease such as needle tip dieback, chlorotic foliage, or reduced growth (James 1984b; James 1984c). Seedlings were carefully extracted from Leach® pine containers and their roots washed thoroughly under running tap water to remove adhering soil. For the seedlings sampled during 10/87 (table 1), soil was collected from the washings in cheesecloth for later analysis. For each seedling, 10 lateral roots were randomly selected and aseptically severed from the root system. Root tips (about 5 mm in length) were cut from each selected lateral root and placed on an agar medium selective for *Fusarium* (Komada 1975). From the sampled lateral roots, five were selected and 5 mm pieces cut from where they joined other roots (joints). These pieces were likewise placed on Komada's medium. All plates were incubated at about 25 degrees C for 7 days under diurnal cycles of cool fluorescent light. *Fusarium* isolates emerging from root tips were transferred to potato dextrose agar and carnation leaf agar and identified using a standard taxonomic guide (Nelson et al. 1983). Percentage of root tips and joints colonized by *Fusarum* and *Trichoderma* spp., common antagonists of *Fusarium* (Papavizas 1985) which also readily grow on Komada's medium, were calculated.

Seedlings were measured (height from the groundline to the tip of the terminal bud and caliper just above the groundline) and the oven-dry weight of the above-ground portion determined. Simple linear regressions were conducted comparing seedling size and biomass parameters with level of root infection to determine if there were significant correlations. All percentages underwent arc-sin transformations prior to analysis.

For seedlings sampled during 10/87 (table 1), analysis of Leach® pine cells and soil adhering to seedling roots and the inner wall of containers were conducted. The bottom 10 mm of each Leach® pine cell was aseptically severed and divided into four pieces of approximately equal size. These pieces were then placed inside surface down on Komada's medium and incubated as described above.

Table 1.--Seedlots selected for analyses of *Fusarium* on the roots of nondiseased containerized ponderosa pine seedlings.

Seedlot	Ranger District	National Forest	Date(s) samples collected	Number of seedlings sampled
0507	Swan Lake	Flathead	10/87	5
0567	Seeley Lake	Lolo	5/87; 8/87	4
2358	Fernan	Idaho Panhandle	10/87	5
2899	Superior	Lolo	5/87; 8/87	4
6227	Sandpoint	Idaho Panhandle	5/87; 8/87	4
6232	Elk City	Nez Perce	5/87; 8/87	4
6417	Sula	Bitterroot	10/87	5
6425	Stevensville	Bitterroot	5/87; 8/87	4
6493	Swan Lake	Flathead	10/87	5
6551	Musselshell	Lewis & Clark	10/87	5

The inside of each Leach® pine cell was rinsed with 25 ml of sterile water and particles of soil and organic matter from the wall were collected in cheesecloth. Together with soil from root washings (see above), these samples were air-dried for 24 hours and subjected to standard soil dilutions to determine colony-forming units (cfu) of *Fusarium* per gram of soil. This procedure required that 0.5 g of each soil/organic matter mixture be combined with 100 ml of 0.3 percent water agar. After thorough mixing, 1 ml of the solution was syringed onto Komada's medium and incubated as described above. The number of *Fusarium* colonies arising from the plates was determined and cfu/gm were calculated.

RESULTS AND DISCUSSION

More than 90 percent of the nondiseased ponderosa pine seedlings sampled had roots that were infected with *Fusarium* (table 2). In eight of the 10 seedlots, all sampled seedlings were infected. Colonization of root tips with *Fusarium* was more common than on other portions of roots, confirming previous findings (James 1986b; James et al. 1987; James, Gilligan and Reedy 1988). Relatively large portions of sampled root systems were colonized, even though colonized roots usually lacked nectoric lesions or other indications of pathogenesis. Only three of the five seedlots sampled for *Trichoderma* had seedlings with roots infected with these fungi. Roots were colonized to a much lesser extent by *Trichoderma* than by *Fusarium*. This was just the reverse of what was found when sampling roots of nondiseased bareroot Douglas-fir seedlings at the nursery (James and Gilligan 1988b). In most previous investigations (James 1986b; James and Gilligan 1988b; James et al. 1987; James, Gilligan and Reedy 1988), roots with a high percentage of colonization by *Trichoderma* were usually colonized to a much lesser extent by *Fusarium*. This may be due to antagonism within the seedling rhizosphere (Papavizas 1985) or prior occupation of root cortical cells by *Trichoderma*. In any event, presence or absence of *Trichoderma* on the roots of containerized conifer seedlings appears to be a good inverse indicator of presence or absence of *Fusarium*.

Table 2.--Colonization of roots of nondiseased containerized ponderosa pine seedlings with *Fusarium* and *Trichoderma* spp. from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	- - - - - - - - - % *Fusarium* Colonization - - - - - - - - -				- - - - - % *Trichoderma* Colonization - - - - -			
Seedlot	Seedling infection	Root tips	Other root pieces	Total root colonization	Seedling infection	Root tips	Other root pieces	Total root colonization
0507	60.0	100.0	46.7	82.2	60.0	13.3	6.7	11.1
0567	100.0	71.1	60.0	69.3	– 3/	--	--	--
2358	60.0	30.0	0.0	20.0	60.0	6.7	6.7	6.7
2899	100.0	82.0	20.0	71.7	--	--	--	--
6227	100.0	86.0	10.0	73.3	--	--	--	--
6232	100.0	72.0	40.0	66.7	--	--	--	--
6417	100.0	68.0	12.0	50.7	40.0	30.0	0.0	20.0
6425	100.0	90.0	40.0	81.7	--	--	--	--
6493	100.0	92.0	32.0	72.0	0.0	0.0	0.0	0.0
6551	100.0	98.0	32.0	76.0	0.0	0.0	0.0	0.0
Averages	91.1	73.6	27.7	62.1	32.0	15.0	5.0	11.7

1/ See Table 1 for seedlot descriptions.

2/ Percentage colonization of infected seedlings only.

3/ Denote data not obtained - not included in averages.

Seedling height, caliper, and oven-dry weight are summarized in table 3. Very poor correlations existed between seedling height or oven-dry weight and extent of root colonization by *Fusarium* (table 4). The correlation between seedling caliper and root colonization was somewhat better, but still not very strong. In this study, it appears that colonization of roots by *Fusarium* did not adversely affect seedling growth or biomass production.

Table 3.--Height, caliper, and oven-dry weights of above-ground portions of nondiseased containerized ponderosa pine seedlings sampled for root colonization by *Fusarium*.

Seedlot	Avg. hgt. (mm)	S.D.*1/*	Avg. caliper (mm)	S.D.*1/*	Avg. oven-dry weight (gms)	S.D.*1/*
0507	126.2	28.3	3.0	0.0	1,410	0.336
0567	158.0	13.2	1.9	0.3	1,400	0.424
2358	162.6	22.1	3.0	0.0	1,662	0.386
2899	181.5	19.1	2.5	0.6	1,775	0.222
6227	158.8	20.8	2.5	0.6	1,800	0.183
6232	143.8	32.7	2.3	0.5	1,600	0.245
6417	153.8	20.1	3.0	0.0	1,934	0.326
6425	138.0	18.9	2.1	0.6	1,225	0.299
6493	190.6	27.9	3.0	0.0	1,577	0.421
6551	141.6	17.9	3.0	0.0	2,008	0.304

1/ S.D. = Standard deviation.

Table 4.--Simple linear regressions comparing seedling height, caliper, and above-ground oven-dry weight with percentage root colonization by *Fusarium*. *1/*

	R^2 *2/*	Equation
Height (x) vs. avg. root colonization (y)	0.002	y = 45.65 + 0.05 x
Caliper (x) vs. avg. root colonization (y)	0.16	y = 106.21 - 20.00 x
Oven-dry wgt. (x) vs. avg. root colonization	0.04	y = 75.83 - 13.94 x

1/ All percentages underwent arc-sin conversions prior to analysis.

2/ Coefficients of determination.

Data for Leach® pine cell colonization by *Fusarium* and *Trichoderma* as well as populations of *Fusarium* assayed in root and inner container wall washings are summarized in table 5. Most sampled containers were colonized with *Fusarium*. In many cases, seedlings which had most of their roots infected with *Fusarium* likewise had their containers infected (figure 1). Usually, the same *Fusarium* isolate colonized both roots and containers. When *Fusarium* colonized the entire sampled piece of container, few other fungi were detected (figure 2), probably indicating occurrence of many propagules on containers. The one exception was when container pieces were colonized with *Trichoderma* (figure 3). As with seedling roots, when containers were extensively colonized with *Trichoderma*, less *Fusarium* was detected.

Table 5.--Occurrence of *Fusarium* and *Trichoderma* within peat-vermiculite soil mix and Leach® pine cells from nondiseased containerized ponderosa pine seedlings from the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

	- - - - - - - - Percent Fusarium Colonization - - - - - - - -				- - - - - Percent Trichoderma Colonization - - - - -	
Seedlot 1/	Container infection	Colon. 2/ intensity	Container 3/ wall colon.	Root 4/ washings	Container infection	Colon. 2/ intensity
0507	60.0	83.3	6,520	8,120	60.0	58.3
2358	60.0	41.7	400.	1,880	100.0	80.0
6417	100.0	80.0	1,600.	2,650	40.0	75.0
6493	100.0	100.0	10,000.	12,040	20.0	100.0
6551	100.0	90.0	4,080.	17,240	20.0	100.0
All lots average	84.0	82.1	4,520.	8,386	48.0	72.9

1/ See Table 1 for seedlot descriptions.

2/ Percentage of colonization of cells infected with Fusarium and/or Trichoderma (infected cells only).

3/ Colony-forming units per gram of soil adhering to the inside walls of containers.

4/ Colony-forming units per gram of soil mix washed from roots.

Because of budget constraints, only a limited number of photos are available.

Figure 1.--Colonization of root pieces (left) of Leach® pine cells (right) by *Fusarium*. Usually the same *Fusarium* isolate colonized roots and the container of each seedling.

Figure 2.--Colonization of a piece of Leach® pine cells by *Fusarium*. When resulting colonies were extensive, few other fungi were detected.

Figure 3.--Colonization of Leach® pine cells with *Trichoderma*. Note the powdery green appearance of *Trichoderma* colonies. The bottom sample is colonized by both *Trichoderma* and *Fusarium*.

Populations of *Fusarium* within soil washed from roots and the inner surface of containers varied among samples, but were generally high for most seedlings (table 5). Levels above about 1,000 cfu/gm are generally considered high enough in field soil for disease to be important. *Fusarium* propagules were generally at greater concentrations on or near the roots than on the outside of the plug adjacent to the wall of the container. Such high *Fusarium* populations would account for the extensive root colonization that was found.

Eighty-two percent of the *Fusarium* isolates obtained from nondiseased ponderosa pine seedlings were *F. oxysporum* Schlect. This is usually the most common species encountered on containerized seedlings (James and Gilligan 1984; James and Gilligan 1985; James, Gilligan and Reedy 1988) and it has been identified as a major pathogenic species in forest tree nurseries (Bloomberg 1971; Bloomberg and Lock 1972). Different strains of *F. oxysporum* exist (Bloomberg and Lock 1972; James 1985c; James and Gilligan 1984; James et al. 1987), including some which are highly pathogenic and others that are mostly saprophytes. The other *Fusarium* species isolated was *F. sporotrichioides* Sherb. This species has previously been isolated from ponderosa pine seed and seedlings (James 1985b), but its role in pathogenesis is unknown.

Results of this investigation indicate that *Fusarium* spp. are very common colonizers of the roots of nondiseased containerized ponderosa pine seedlings. These fungi also readily colonize the soil mix adjacent to roots and the Leach® pine containers within which seedlings are grown. Other investigations (James and Gilligan 1988a) have indicated that standard container-cleaning techniques may not adequately remove *Fusarium* inoculum. Therefore, it is likely that inoculum increases when containers are used for several successive crops. However, large amounts of inoculum do not necessarily adversely affect seedling health. *Fusarium* isolates from nondiseased seedlings are capable of causing disease (James et al. 1988). Why they do not always do so is unknown. This study indicates that it is not because there is insufficient inoculum. Other factors such as level of host stress may be most important in disease manifestation.

When containerized seedlings are lifted, stored, and outplanted, they are probably stressed significantly. This may render them more susceptible to disease from the *Fusarium* occupying their roots, although this has not been proved. It is possible that seedlings whose roots are extensively colonized with *Fusarium* are less likely to survive outplanting. Tests are currently underway to evaluate the fate of *Fusarium* on outplanted containerized Douglas-fir seedlings. Outplanted seedlings may die as a result of *Fusarium* root disease (James 1987a). However, importance and extent of this occurrence has yet to be determined.

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